

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P49923PC00	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/NL 00/ 00440	International filing date (day/month/year) 23/06/2000	(Earliest) Priority Date (day/month/year) 24/06/1999
Applicant NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURW...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

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☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00440

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 12 is directed to a method of surgery practiced on a non-human mammalian test animal, the search has been carried out and based on the alleged effects of said method.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-13 (all partially)

A test animal useful in an animal model system for atherosclerosis wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels; said test animal which is an ApoE-/- mouse; A method for accelerating the onset and/or development of atherosclerotic phenomena in a non-human mammalian test animal comprising applying a vessel-restricting device to at least one of its blood vessels; Use of a non-human mammalian test animal susceptible to the induction of atherosclerosis and carrying a vessel-restricting device applied to at least one of its blood vessels, for identifying substances, diets or treatments having an anti-atherosclerotic effect.

2. Claims: 1-13 (all partially)

As for subject 1, but wherein said test animal is an LDL-R-/- mouse

3. Claims: 1-13 (all partially)

As for subject 1, but wherein said test animal is an ApoE3 mouse

INTERNATIONAL SEARCH REPORT

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Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

To the original abstract the following phrase is added:

Said test animal is preferably an Apo-E-deficient mouse,
an LDL-R-deficient mouse or an Apo-E3 Leiden transgenic mouse.

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REC'D 12 NOV 2001

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P49923PC00		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) FOR FURTHER ACTION	
International application No. PCT/NL00/00440	International filing date (day/month/year) 23/06/2000	Priority date (day/month/year) 24/06/1999	
International Patent Classification (IPC) or national classification and IPC A01K67/02			
Applicant NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURW...			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 23/01/2001	Date of completion of this report 05.11.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Barnas, C Telephone No. +49 89 2399 7469 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00440

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-13 as originally filed

Claims, No.:

2-5,7-13 as originally filed

1,6 as received on 24/09/2001 with letter of 24/09/2001

Drawings, sheets:

1-4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/NL00/00440

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5, 6, 11
	No:	Claims	1-4, 7-10, 12, 13
Inventive step (IS)	Yes:	Claims	5, 6
	No:	Claims	11
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

- 2. Citations and explanations**
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

2. Art. 33(3) PCT, Inventive Step

Prompted by the need for treatments for atherosclerosis, the skilled person uses the rabbits of D1 for screening substances, diets and treatments to identify having anti-atherosclerotic effect. **Claim 11** is, therefore, not inventive.

3. Additional Observations:

The cited prior art does not contain any indication that would prompt the person to provide a transgenic mouse as described in claims 5 and 6 with a restricting device. Claims 5 and 6 seem, therefore, inventive (see however, F VIII).

Re Item VIII

Certain observations on the international application

1. D2 and D3 describe transgenic mice with a disorder promoting their susceptibility to atherosclerosis which are provided with a vessel restricting cuff. Said animals, however, do not seem to exhibit an accumulation of foam cells. Thus, not all as described in claim 5 necessarily exhibit accumulation of foam cells. Amer 1 defines its subject matter, therefore, by the result to be achieved ("... and exhibit accumulation of foam cells..."). The features, however, which indicate achieve said result are missing in said claim. Because of the lack of said features are considered essential claim 5 is not clear (Art. 6 PCT).

2. According to the teaching of claim 6, ApoE^{-/-}, LDL-R^{-/-} and ApoE3 Leiden mice having a cuff exhibit accumulation of foam cells. In the description of the prior application, however, only ApoE3 Leiden mice are shown to exhibit such an accumulation. Because of the disclosure of D1 and D2 (see above, paragraph 1) cannot be assumed that any transgenic mice with a disorder promoting their susceptibility to atherosclerosis having a vessel restricting cuff exhibit an accumulation of foam cells. In view of D1 and D2, the teaching of claim 6 is, therefore, not as far as referring to ApoE^{-/-} and LDL-R^{-/-} mice (Art. 6 PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- D1: SOMA M ET AL: 'Recombinant apolipoprotein A-I/Milano dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits' CIRC RES, vol. 76, no. 3, March 1995 (1995-03), pages 405-411, XP000857882 cited in the application
- D2: MOROI M ET AL: 'Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice' J. CLIN. INVEST., vol. 101, no. 6, 15 March 1998 (1998-03-15), pages 1225-1232, XP002126586 cited in the application
- D3: WO 97 33989 A (GEN HOSPITAL CORP) 18 September 1997 (1997-09-18)

1. Art. 33(2) PCT, Novelty

1.1. D1 describes rabbits with a collar around the carotid artery which were fed with a cholesterol rich diet. Said rabbits develop neointimal hyperplasia which is enriched by cholesterol-loaded macrophages, i.e. foam cells (see p. 408, left column and right column, last paragraph, last sentence). Although said foam cells represent only 10% of the total cells in the neointima, the enrichment of these cells nevertheless represents a (weak) accumulation at the location of the collar.

1.2. Because the rabbits of D1 show the development of neointima formation they mimic a feature of human atherosclerosis (see D2: p. 1225, left column, last line - right column, line 1 and right column paragraph 2, ln. 1-5). Since the rabbits of D1 have the potential to develop a phenomena associated with atherosclerosis, they are "susceptible to the induction of atherosclerosis" (see the specification, p. 5, paragraph 3, ln. 2-4, in this context) and "useful in an animal model system for atherosclerosis".

D1 is, therefore, novelty destroying for **claims 1-4**.

1.3. The neointimal hyperplasia in the rabbits of D1 could be inhibited by treatment with rA-I_M/A-I_M-containing rHDL. Said document is, therefore, further novelty destroying for **claims 7-10, 12 and 13**.

24-09-2001

Your ref. PCT/NL00/00440
Our ref. Ln/P49923PC00
Date 24-09-01

Patent Attorneys
Trademark Attorneys
Attorneys-at-Law

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VEREENIGDE

NEW CLAIMS 1 AND 6

1. A test animal useful in an animal model system for atherosclerosis, wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels and as a result thereof exhibits an accumulation of foam cells in said blood vessel at the location of the device.
6. A test animal according to claim 5, wherein said transgenic mouse is selected from the group consisting of ApoE^{-/-}, LDL-R^{-/-} and ApoE3 Leiden mice.

EPO - DG 1

24. 09. 2001

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(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 January 2001 (04.01.2001)

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(10) International Publication Number
WO 01/00014 A3

(51) International Patent Classification⁷: **C12N 15/12**,
A01K 67/027, A61K 49/00, C07K 14/775, 14/705

(74) Agent: PRINS, Ir, A., W.; Vereenigde, Nieuwe Parklaan
97, NL-2587 BN The Hague (NL).

(21) International Application Number: PCT/NL00/00440

(22) International Filing Date: 23 June 2000 (23.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99202051.1 24 June 1999 (24.06.1999) EP

(71) Applicant (for all designated States except US): NED-
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(NL).

(72) Inventors; and

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Jan-Willem, Henricus, Pieter [NL/NL]; Stieltjesstraat
13, NL-2313 SH Leiden (NL).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
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IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG,
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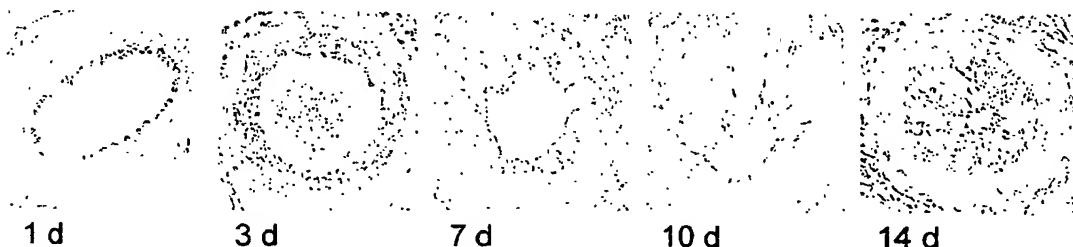
Published:

— with international search report

(88) Date of publication of the international search report:
27 December 2001

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: TEST ANIMAL FOR ATHEROSCLEROSIS MODEL AND METHODS FOR TESTING AND SCREENING



(57) Abstract: A test animal useful in an animal model system for atherosclerosis, wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels. A method for testing the (anti-)atherosclerotic effect of a substance, diet or treatment in an animal model system for atherosclerosis, comprising subjecting a test animal according to the invention to a test treatment with the substance, diet or treatment to be tested and analyzing the (anti-)atherosclerotic effect, if any, on the blood vessel restricted by the vessel-restricting device. The method may be used for screening potentially useful substances, diets or treatments.

WO 01/00014 A3

INTERNATIONAL SEARCH REPORT

International Application No

P NL 00/00440

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C12N15/12 A01K67/027 A61K49/00 C07K14/775 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01K A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 33989 A (GEN HOSPITAL CORP) 18 September 1997 (1997-09-18) page 6, line 7-30; claims 7,10; figures 16,17; examples 13,15	1-13
X	MOROI M ET AL: "Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice" J. CLIN. INVEST., vol. 101, no. 6, 15 March 1998 (1998-03-15), pages 1225-1232, XP002126586 cited in the application	1-5,7-9, 12,13
Y	figure 1	6,10,11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

5 January 2001

Date of mailing of the international search report

22.01.2001

Name and mailing address of the ISA

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Authorized officer

Lonnoy, O

INTERNATIONAL SEARCH REPORT

International Application No

P 00/00440

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	✓ SOMA M ET AL: "Recombinant apolipoprotein A-IMilano dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits" CIRC RES, vol. 76, no. 3, March 1995 (1995-03), pages 405-411, XP000857882 cited in the application figure 2	1-4,7-13
Y	✓ VAN VLIJMEN B ET AL: "Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice" J. CLIN. INVEST., vol. 93, no. 4, April 1994 (1994-04), pages 1403-1410, XP000857884 cited in the application table 2	6,10,11
Y	✓ BRESLOW J: "Mouse models of atherosclerosis" SCIENCE, vol. 272, no. 5262, 3 May 1996 (1996-05-03), pages 685-688, XP002126587 table 1	6,10,11
A	✓ WO 97 05247 A (UNIV DUKE) 13 February 1997 (1997-02-13)	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

P/NL 00/00440

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9733989	A	18-09-1997	NONE		
WO 9705247	A	13-02-1997	US	5767337 A	16-06-1998
			AU	720830 B	15-06-2000
			AU	6485096 A	26-02-1997
			EP	0842271 A	20-05-1998
			JP	11510052 T	07-09-1999

INTERNATIONAL SEARCH REPORT

International application No.
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2. ☐ Claims Nos.:
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3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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1. Claims: 1-13 (all partially)

A test animal useful in an animal model system for atherosclerosis wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels; said test animal which is an ApoE-/- mouse; A method for accelerating the onset and/or development of atherosclerotic phenomena in a non-human mammalian test animal comprising applying a vessel-restricting device to at least one of its blood vessels; Use of a non-human mammalian test animal susceptible to the induction of atherosclerosis and carrying a vessel-restricting device applied to at least one of its blood vessels, for identifying substances, diets or treatments having an anti-atherosclerotic effect.

2. Claims: 1-13 (all partially)

As for subject 1, but wherein said test animal is an LDL-R-/- mouse

3. Claims: 1-13 (all partially)

As for subject 1, but wherein said test animal is an ApoE3 mouse



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